OVARIAN MATURATION INDUCED BY EYESTALK ABLATION IN PINK SHRIMP, Penaeus duorarum BURKENROAD[1]

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ABSTRACT

This paper describes attempts to induce maturation of ovaries and ova in pink shrimp (Penaeus duorarum) by eyestalk ablation and by control of environmental variables (light, temperature, salinity, and food). Ovaries and ova of pink shrimp can be induced to ripen within 2 weeks by eyestalk ablation.

INTRODUCTION

In a review on the culture of penaeid shrimps of the Gulf and Caribbean region, Webber (1970) indicated that continued increase in demand and value of these shrimps would provide considerable economic incentive toward further development of methods to culture them in captivity. He emphasized that methods developed so far for rearing large numbers of Penaeus spp. in captivity still depend upon capture of gravid females from wild stocks as a source of fertilized ova. Though Japanese workers are said to have reared multiple generations of one species, Penaeus japonicus, in captivity, and indications are that other species (e.g., P. occidentalis) develop mature ovaries in captivity (Dr. Eric Heald, personal communication), methods to induce maturation of shrimp will be

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required for these and other Penaeus spp. to provide year-round control over reproduction. Control of the reproductive cycle of Penaeus spp. in captivity also would make possible the investigation of genetic improvement and would make shrimp culture independent of wild stocks.

The pink shrimp (Penaeus duorarum Burkenroad) is among the three most important commercial shrimps in the United States (Williams, 1965), and the commercial shrimp fishery of southern Florida is based upon it (Saloman, et al., 1968). Tabb, et al. (1972) developed methods for mass production of postlarval pink shrimp, after initial attempts to rear the larval stages in captivity were successful (Dobkin, 1961; Ewald, 1965). Postlarval pink shrimp obtained in this way have been reared to marketable size (Caillouet, et al., 1972) at the Turkey Point, Florida, laboratory (Tabb, et al., 1969).

The problem of closing the life cycle of pink shrimp in captivity is one of inducing the females to mature. Male pink shrimp reared from ova mature in captivity, since they can be found to contain spermatophores visible through the carapace, and spermatophores excised from such males contain spermatozoa. Though female pink shrimp reared in captivity from ova have not been observed to develop mature ovaries (except following eyestalk removal), their ovaries do contain immature ova.[3] This is also true of female pink shrimp collected from estuarine waters of Tampa Bay (Eldred, 1958) and Everglades National Park (Cummings, 1960) in Florida. Thus oogenesis in pink shrimp begins during the estuarine phase of the life cycle in Florida, but maturation of the ovaries and ova is apparently not completed until the shrimp leave the estuaries to migrate to the offshore spawning grounds. Pink shrimp have been reported to copulate in captivity (Eldred, 1958), and this is further corroborated by presence of spermatophores in a large proportion of the females grown in ponds at Turkey Point.

Lee and Lee (1970) showed that oogenesis in P. japonicus was initiated prior to the emigration of the shrimp to deeper water in December and that vitellogenesis (yolk deposition in the ova) bebegan in May of the following year. In decapod crustaceans vitellogenesis is influenced by hormones (Adiyodi and Adiyodi, 1970). Within each eyestalk a gonad inhibiting hormone (GIH) is secreted by the medulla terminalis ganglionic X-organ (MTGX) and is stored in the sinus gland. The X-organ has been identified in P. setiferus (Young, 1959). The Y-organ which secretes the molt hormone, crustecdysone, also has an influence on maturation, and a gonad stimulatory hormone (GSH) apparently originates in the central

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[3]For simplicity, the terms ovum and ova are used to refer to stages of development of ovarian eggs. It is recognized that, in some cases, the terms oocyte and ootid might be more precise, but their proper use requires detailed knowledge of the progress of meiotic divisions accompanying oogenesis.

central nervous system (Adiyodi and Adiyodi, 1970). Ablation (surgical removal) of eyestalks has been shown to accelerate vitellogenesis in many decapod crustaceans, and since July 1970 I have repeatedly induced maturation of ova and ovaries in pink shrimp in a similar way (Idyll, 1971). Reproductive cycles in marine invertebrates are also influenced by exogenous factors such as food, temperature, light and salinity (Giese, 1959).

The objective of this investigation was to induce maturation in female pink shrimp. The approach is one of attempting to induce maturation of ova and ovaries through manipulation of environmental variables (light, temperature and salinity), diet and hormone balance. Only eyestalk ablation has been successful so far, but this alone may lead eventually to control of shrimp reproduction in captivity through artificial fertilization of ova. The unsuccessful attempts of this investigation should also be of interest to those engaged in similar studies of reproductive physiology in Penaeus spp.

SOURCES OF PINK SHRIMP

The pink shrimp used in this study were either collected from wild stocks or reared from ova at Turkey Point. For those reared from ova, the ova were obtained from gravid females collected from the Tortugas shrimping grounds west of Key West or from the Sanibel shrimping grounds west of Ft. Myers, Florida. Those collected from wild stocks were obtained from Biscayne Bay near Miami and from the Tortugas and Sanibel shrimping grounds. Female shrimp from all these sources have been induced to develop ripe ova and ovaries by eyestalk ablation. Though some experiments have been conducted on smaller shrimp, most of the specimens used have been 100 mm in total length or larger.

SEAWATER LABORATORIES

Three seawater laboratories (Figure 1) provide temperature and light (spectral quality and photoperiod) control for the experiments which are conducted at the Fisher Island laboratory near Miami Beach, Florida. Seawater is re-circulated through oystershell filters, but clean water must be added at intervals to maintain water quality. In initial experiments, the entire bottoms of the wooden tanks (300 ℓ capacity) were covered with calcareous sand, but the sand quickly became contaminated with wastes from the shrimp and left-over food, and anaerobic conditions developed in the sand. To avoid this problem, sand was removed, and clean sand was put into two wooden trays (60 x 38 x 8 cm deep) to a depth of about 5 cm, one tray on each end of the tank, to provide a substrate for the shrimp to burrow into. These trays could easily be removed for cleaning. Later, sub-sand filters (air-lift water circulation) were put into these trays to increase water filtration capacity in each tank and to prevent anaerobism from developing within the sand. Seawater is obtained from the ship channel

bordering Fisher Island, but only during periods of incoming or high slack tide when water quality is best. Salinities at this location are near 30-35 ppt throughout the year.

FIXATION AND EXAMINATION OF OVARIES AND OVA

Methods for determining the degree of maturation of female pink shrimp have been reported (Eldred, 1958; Cummings, 1960, 1961; de Vries and Lefevere, 1966; Burukovski, 1970). Several of these methods were tried with the objective of finding a rapid and reliable method for assessing maturation quantitatively. Pink shrimp collected from the spawning grounds were used for this purpose. Whole shrimp were fixed in Bouin's picro-formol solution after incisions (Figure 2) were made through the dorsal part of the exoskeleton to facilitate penetration of the fixative. Gross external characteristics of fixed ovaries were observed (e.g., color, texture and opacity). Fixed material was treated in three ways:

1) Samples from ovaries were imbedded in paraffin and sectioned at 8-10 μ. Sections mounted on slides were stained with Harris' alum hematoxylin, were counterstained with eosin Y, and were examined with a microscope (Figure 3).

2) Ova (Figure 4) in samples from ovaries were teased apart in a few drops of Bouin's solution on a slide, and the frequency distribution of ovum diameter (measured with ocular micrometer) was determined by the method used by Cummings (1961).

3) A thin cross-sectional slice (with scalpel) of both lobes of a sample of ovary from the anterior part of the first abdominal segment was placed on a slide, and the cross-sectional area of the slice was determined with a dot-grid (4 dots per mm^2) attached to a cover-slip placed on top of the slice. For each slice, area was determined by counting all dots within but not touching the periphery of the section and then multiplying the number of dots by 0.25 mm^2.

4) The stage of development of the largest of the ova in ovarian samples taken from the first abdominal segment and prepared as in 2 above (Figure 4) was determined by microscopic examination as in Cummings (1961). For this purpose, the ovarian sample was taken from either the right or left ovarian lobe randomly.

The first three techniques consumed far too much time to be practical, but the fourth technique, that of assessing the stage of development of the largest of the ova within an ovary sample, could be used rapidly for large numbers of shrimp, so it was used routinely. Gross external characteristics of fixed ovaries provided additional information. When nearly ripe and ripe ova were teased apart on a slide they sometimes ruptured. For this reason, it was not always possible to measure these larger ova. This

problem became evident when large numbers of rod-like bodies (King, 1948; Cummings, 1961; de Vries and Lefevere, 1966) which characterize ripe ova (Figures 3 and 4) were found on slides which apparently otherwise contained only undeveloped and developing ova (the ripe ova had been ruptured and the peripheral rod-like bodies were released into the Bouin's solution). Therefore, when these rod-like bodies were found either in the Bouin's solution or on the periphery of ova in the Bouin's solution on a slide, the sample was classified as one containing ripe ova. The following four developmental stages used in this study are similar to those employed by King (1948) and Cummings (1961):

Stage 1 - largest ova have clear cytoplasm and prominent nuclei - UNDEVELOPED

Stage 2 - largest ova with developing opaqueness of the cytoplasm, and nuclei are less prominent than in stage 1 - DEVELOPING

Stage 3 - largest ova have opaque cytoplasm and obscured nuclei, but peripheral rod-like bodies are absent - NEARLY RIPE

Stage 4 - ova have opaque cytoplasm and obscured nuclei, and peripheral rod-like bodies are present in the oocytes, in Bouin's solution on the slide, or both - RIPE

FREQUENCY DISTRIBUTION OF OVUM DIAMETER

Among the first 114 specimens examined, 24 were selected for ovum diameter-frequency analysis. Ovaries from the 24 specimens were first classified according to the developmental stage of the largest of the ova (Figures 5-8), then the frequency distribution of ovum diameter was determined as in Cummings (1961). Since all stages contained large numbers of stage 1 ova (undeveloped, less than 0.1 mm in diameter), this group of small ova was often excluded from the sample measurements (Figures 5-8). Frequency distributions based upon sample sizes between 200 and 500 ova did not differ significantly[4] (Figure 6, specimen 34; Figure 7, specimens 35 and 36), and sample size of 100 ova seems adequate (Figure 7, specimen 41) to describe an ovum diameter-frequency distribution. No significant differences were detected in frequency distribution of ovum diameter among three regions (anterior, middle and posterior) of the abdominal lobes of the ovary (Figure 7, specimen 41). Variation among specimens assigned to a particular stage and overlap in ovum diameter-frequency distributions among the stages (Figures 5-8) are measures of variation in the method of classifying ovaries based upon characteristics of the largest ova. Such

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[4]Used throughout to indicate the 95 percent level of confidence.

variation does not influence classification of ripe ova which are easily distinguished from the other stages qualitatively; i.e., by the presence of peripheral rod-like bodies.

OVARY CROSS-SECTIONAL AREA AND OVUM STAGE

The relationship between ovary cross-sectional area and developmental stage of the largest of the ova in ovarian samples from the first abdominal segment of both normal and ablated female pink shrimp was determined by simple correlation analysis. Significant positive correlations were detected between these two measures of maturation for both normal and ablated shrimp (Table 1). Both measures of maturation exibited significant positive correlations with shrimp size as measured by carapace length (from ocular groove to posterior dorsal edge of carapace).

EYESTALK ABLATION

For ablation experiments, eyestalks were removed with small surgical scissors. They were severed near the base, and the wound was immediately cauterized with a pencil-type soldering iron with a chisel-shaped tip in order to prevent loss of hemolymph. It is suspected that the central nervous system may have at times been affected by cautery, since the shrimp would sometimes swim in a spiral fashion. However, it is believed that ovarian maturation was primarily influenced by removal of the eyestalks, since Thomas E. Jannke (personal communication) has obtained similar ovarian maturation by ligating the eyestalks at the base before severing them. The ovaries usually ripened within two weeks after ablation of eyestalks. Eyestalk ablation also seemed to precipitate molting, since animals usually molted within a few days. However, this observation should be evaluated in light of the possible influence of handling on molting in shrimp. Though survival of ablated shrimp was lower than that of normal shrimp (example, Figure 9), some ablated shrimp have lived more than 45 days following the operation. Survival in ablated shrimp is enhanced if the water is cooled to 15 to 20 C the day before ablation and held at that temperature for at least a day after ablation.

EXPERIMENTS

I have conducted experiments in which light (spectral quality and photoperiod), temperature, salinity and diet were controlled, but the ovaries and ova matured only when eyestalks were removed from the shrimp. Since the experiments are far too numerous to elaborate here, and since none except those in which eyestalks were removed have been successful so far, I will describe them only in general terms in the remaining paragraphs. As an example, results of one experiment are shown in Figure 9. It is possible

that failure of methods other than eyestalk ablation to induce maturation in pink shrimp may have been due to an insufficient testing period. The duration of most of the experiments was 2 months or less, though some have exceeded this. Whether or not these experiments were of insufficient duration will be determined only after pink shrimp are induced to mature in captivity by methods other than eyestalk ablation. In pink shrimp of a size capable of maturing, I suspect that molting and maturation are intimately related and that both can be accomplished within one lunar month. This is merely a working hypothesis based on observations by other investigators that the proportion of gravid females in the catch and abundance of early larval stages seem related to the lunar cycle. Though maturation takes place within 2 weeks after eyestalk removal, this does not necessarily support the working hypothesis, since ablation changes the hormone balance drastically and immediately by removing the source of GIH.

LIGHT

Blue light (about 470 nm, similar to that on the spawning grounds near 30 m depth), green light (about 540 nm) and white light have been used. Colored light was achieved with colored fluorescent lamps covered with colored plexiglas light filters. White fluorescent lamps were used for white lighting. Continuous light, combinations of light and darkness (24 hour cycles), and continuous darkness were tested.

TEMPERATURE AND SALINITY

Temperature and salinity have been controlled to simulate the offshore spawning environment (relatively stable conditions, 25 to 30 C and 35 ppt). Stable conditions of colder temperatures and lower salinities, as well as varying conditions of temperature and salinity have also been included.

DIET

I most frequently have fed diets prepared from commercially available trout foods (e.g., Glencoe Mills, Inc.) and ground squid mantles supplemented with various additives (e.g., beta carotene, phosphatidylcholine, and cholesterol) that are either yolk constituents or were thought to be precursors of yolk. Carotenoids which are important constituents of the yolk of decapod crustacean ova (Cheesman, et al., 1967; Kerr, 1969; Zagalsky, et al., 1967; Zagalsky, et al., 1970; Wallace, et al., 1967) must be obtained from the diet, as is the case with sterols (Florkin and Scheer, 1970). It is of particular interest that both carotenoids and sterols are found in relatively high concentration within the organic carbon of recent sediments in the Gulf of Mexico (Schwendinger and Erdman, 1963, 1964). This observation coupled with the positive correlation between abundance of shrimp and concentrations of organic carbon in the bottom sediments of the Tortugas

shrimping grounds (Grady, 1971) suggests the need for comparison between ovarian lipids and lipids in the sediments on the spawning grounds. Phospholipids seem to be the principal transport moiety in crustaceans (Florkin and Scheer, 1970). William Gehring (personal communication) has used thin-layer chromatography to identify phosphatidylcholine as well as sterol (probably cholesterol), triglycerides, monoglycerides and animo-acid-containing phospholipids from the ovaries of mature pink shrimp. Thus the choice of diet additives appears justified, if for no other reason than to assure that the animals are receiving at least some of the major yolk-building materials. Vitamins E (DL alpha tocopherol) and D_2 (calciferol) have also been added to some diets as suggested by Dr. L. Provasoli (personal communication). Ground beef liver and whole eggs (chicken) have also been used in the earlier diets.

HORMONES

The major follicular hormone of mammals, 17 beta estradiol, has been added to some of the test diets without result, but emphasis is now being placed upon the use of insect juvenile hormones and their analogs (Pfiffner, 1971; Slama, 1971) as possible ovarian stimulants for shrimp. Similarities between insect and crustacean hormones have been demonstrated in the past, and crustecdysone (20-hydroxyecdysone; Kees, 1971), has recently been shown to inhibit maturation of ova in an insect by preventing lipid synthesis (Chamberlain and Barrett, 1971). An antagonism between molting and maturation in decapod crustaceans has long been recognized (Adiyodi and Adiyodi, 1970). It is reasonable to expect similarities in the ovary stimulating hormones of crustaceans and insects. Insect juvenile hormones and their analogs (which are available commercially) have been shown to induce ovum maturation in insects (Pan and Wyatt, 1971; Pfiffner, 1971), and their use for this purpose in crustaceans bears investigation.

PRESSURE

The effect of pressure on maturation in pink shrimp has not been tested in my studies. At depths at which pink shrimp spawn the pressure is near 4 atmospheres. Since maturation was induced by eyestalk ablation in shrimp held at 1 atmosphere (sea level), and since the effect of pressure is more difficult to test than that of other variables tested, I have till now postponed study of the pressure effect. It may be that pressure influences maturation and preparations are being made to test it.

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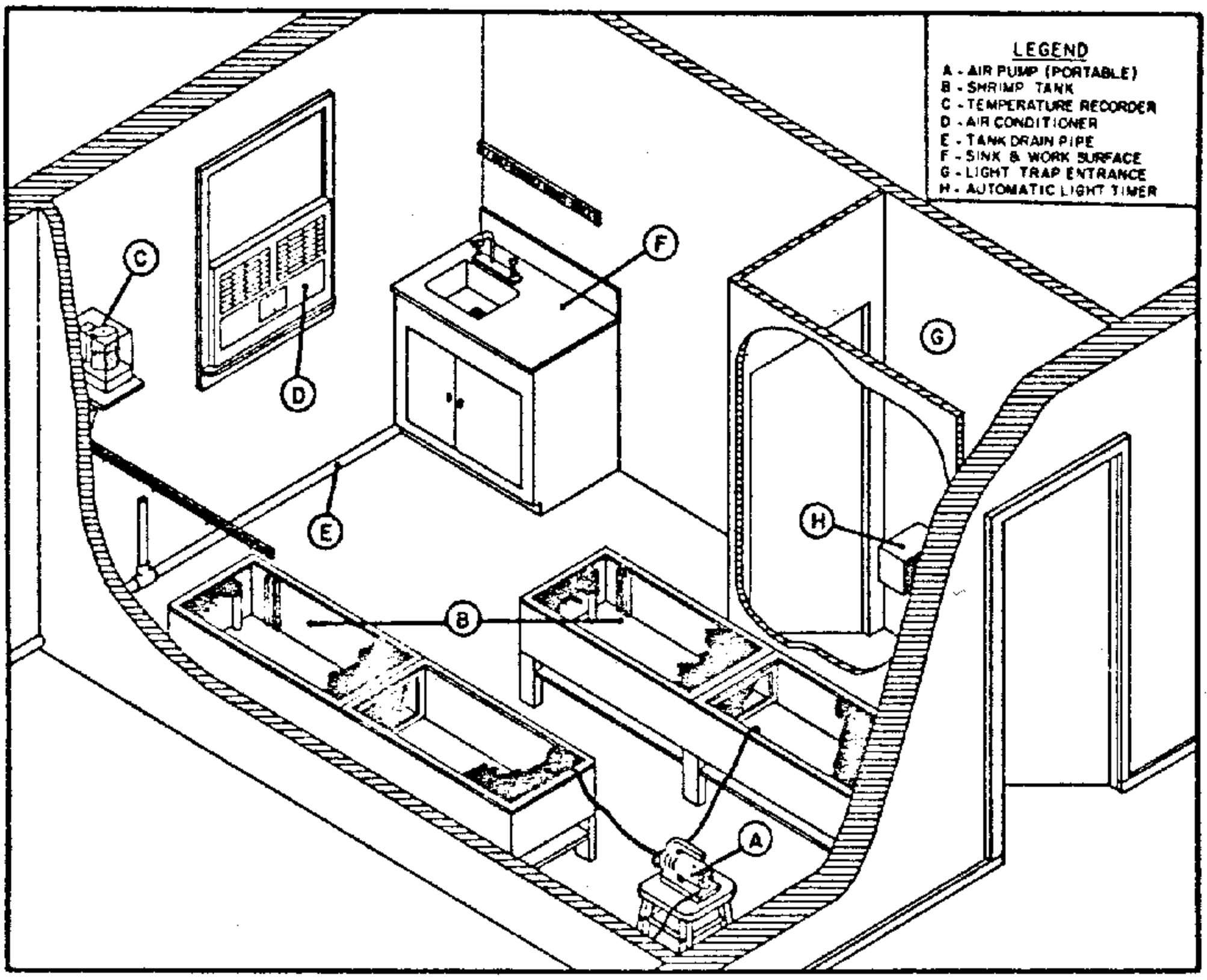

Figure 1. Design of seawater laboratories at Fisher Island, Florida. Wooden shrimp tanks are table-height and are equipped with removable screen covers. Oyster-shell filters external to the tanks are not shown.

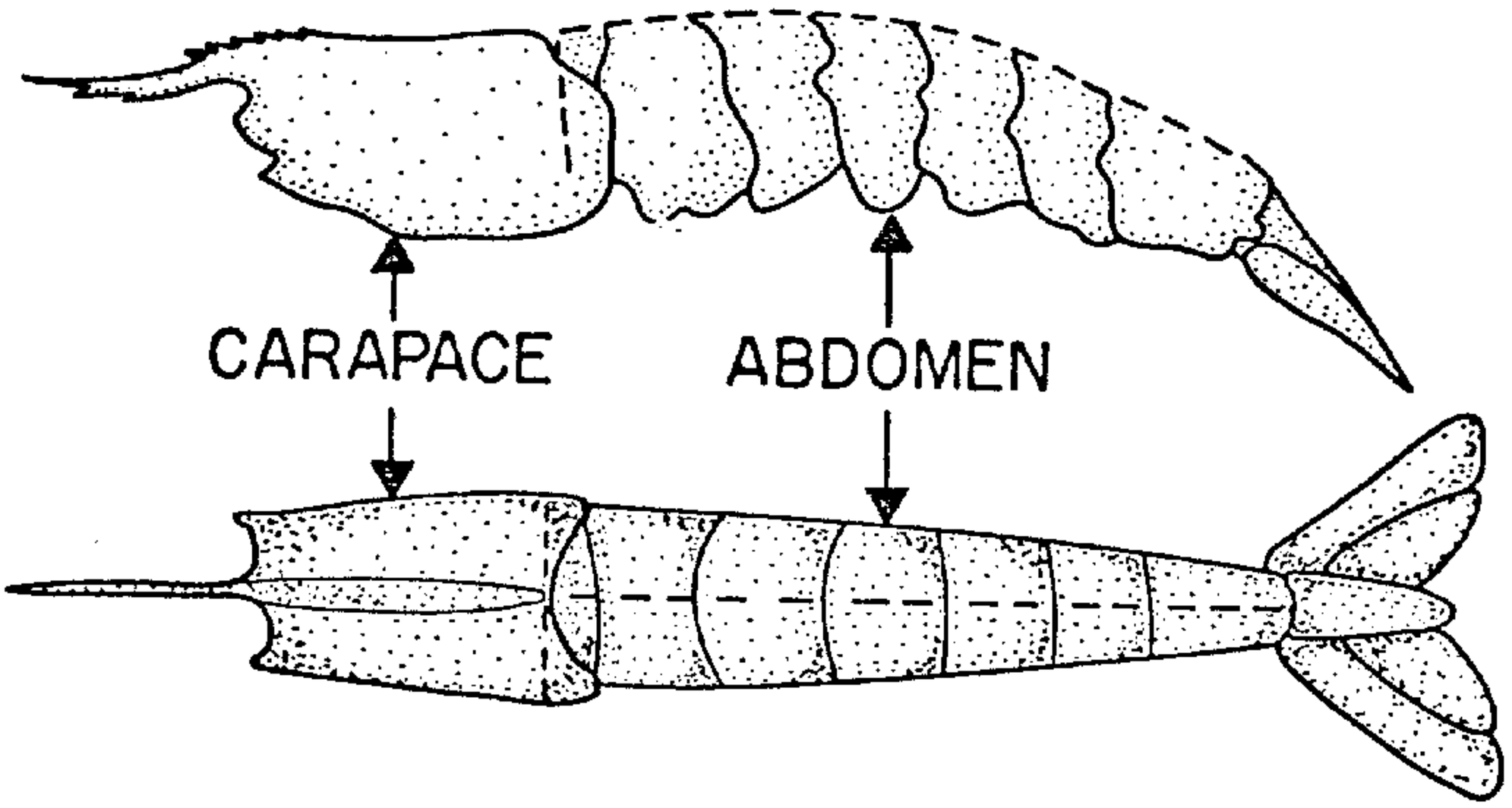

Figure 2. Location of incision (dashed line) through exoskeleton of female shrimp to facilitate penetration of Bouin's picro-formol fixative.

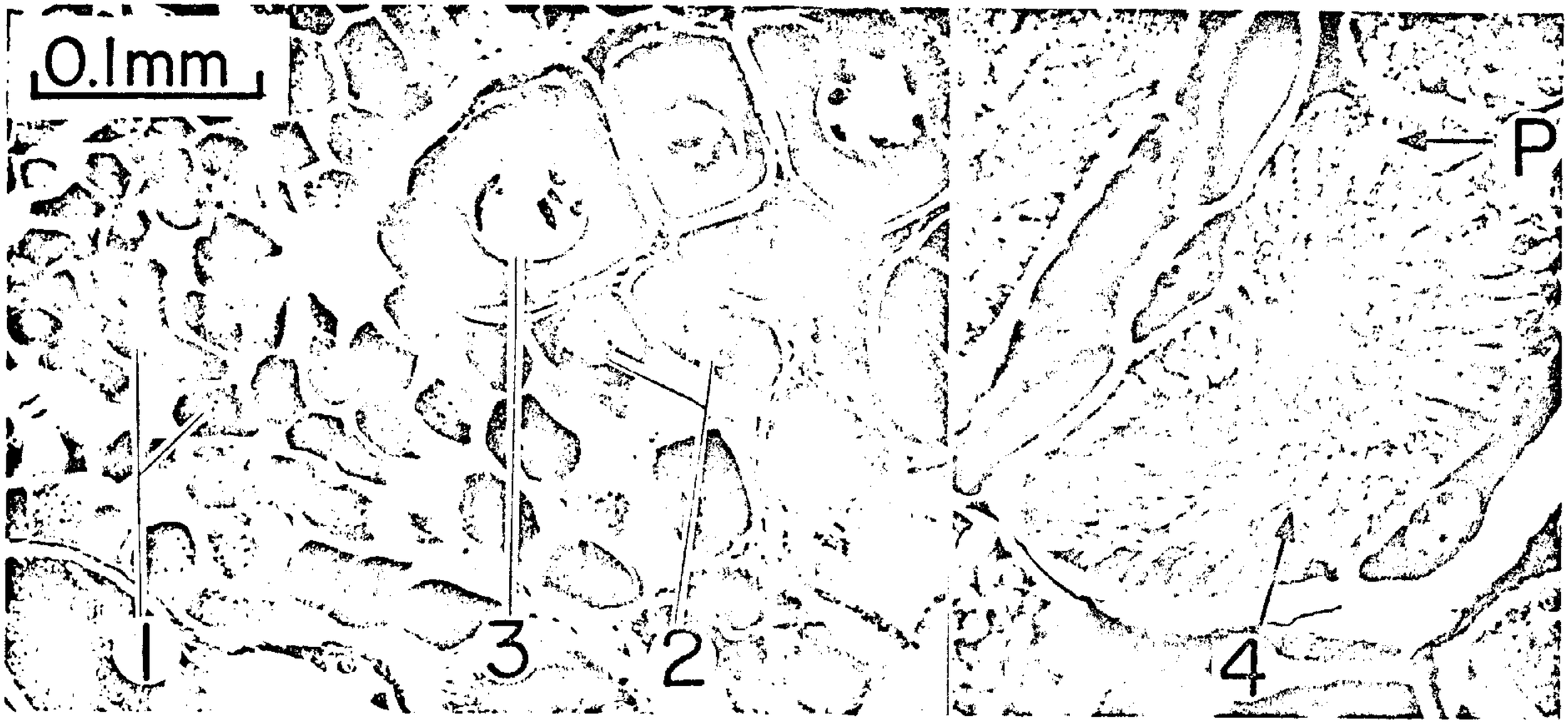

Figure 3. Ovarian sections of a mature pink shrimp. Ovum stages 1 (undeveloped), 2 (developing) 3 (nearly ripe) and 4 (ripe) are shown. The peripheral rod-like bodies (P) characteristic of ripe ova are easily discernible.

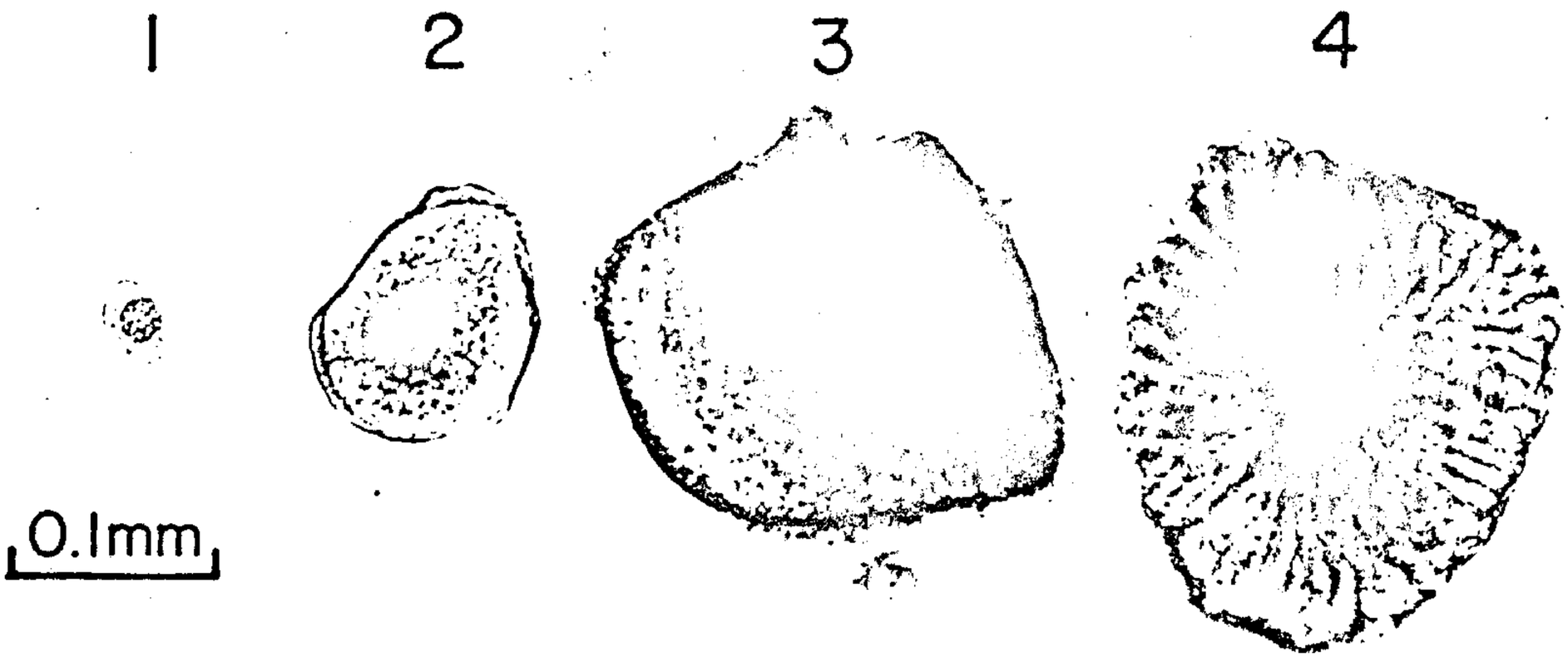

Figure 4. Whole ova, stages 1 (undeveloped), 2 (developing), 3 (nearly ripe) and 4 (ripe), dispersed in Bouin's picro-formol solution on a slide. The peripheral rod-like bodies characteristic of ripe ova are easily discernible in stage 4.

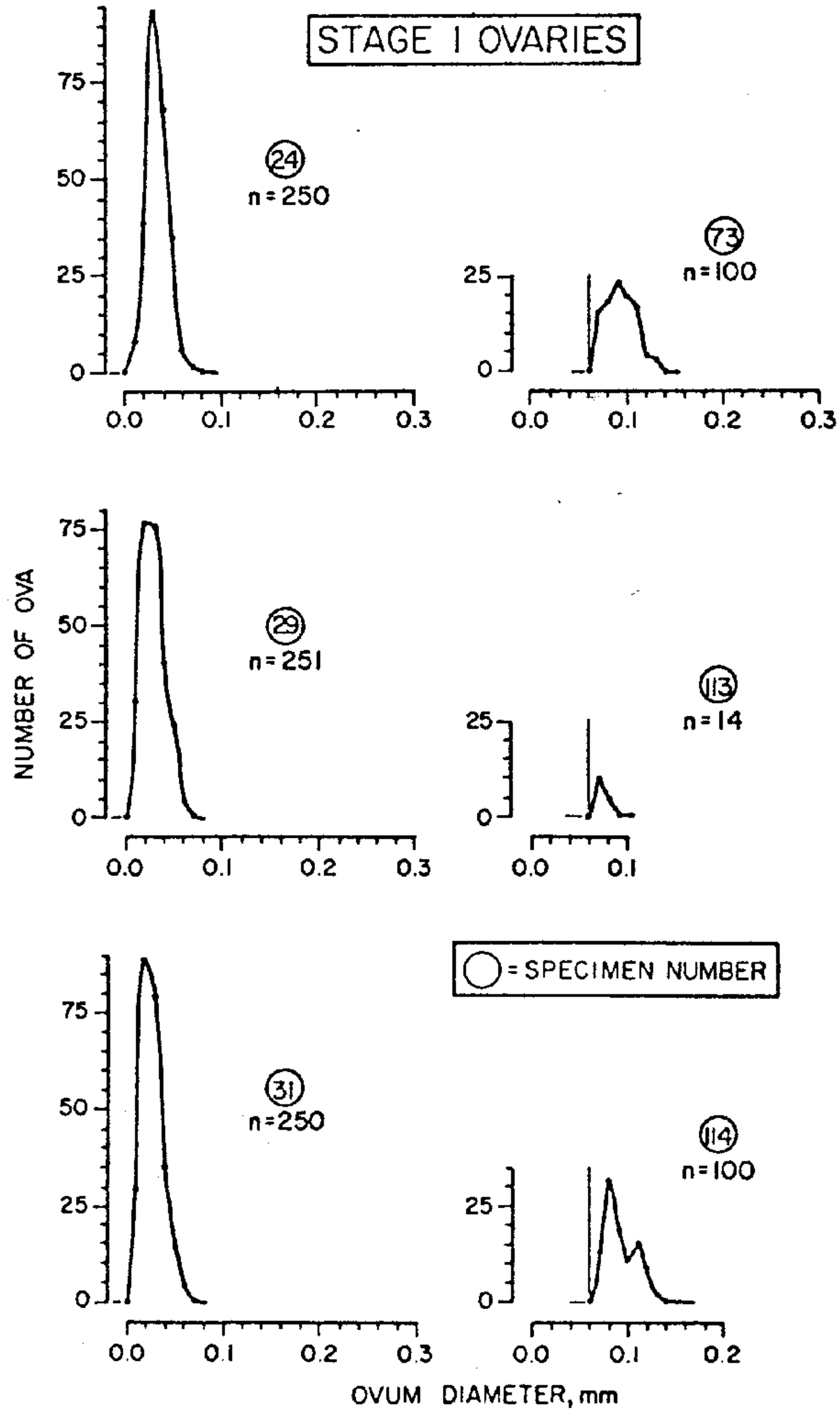

Figure 5. Ovum diameter-frequency distributions of pink shrimp ovary samples classified as stage 1 (undeveloped).

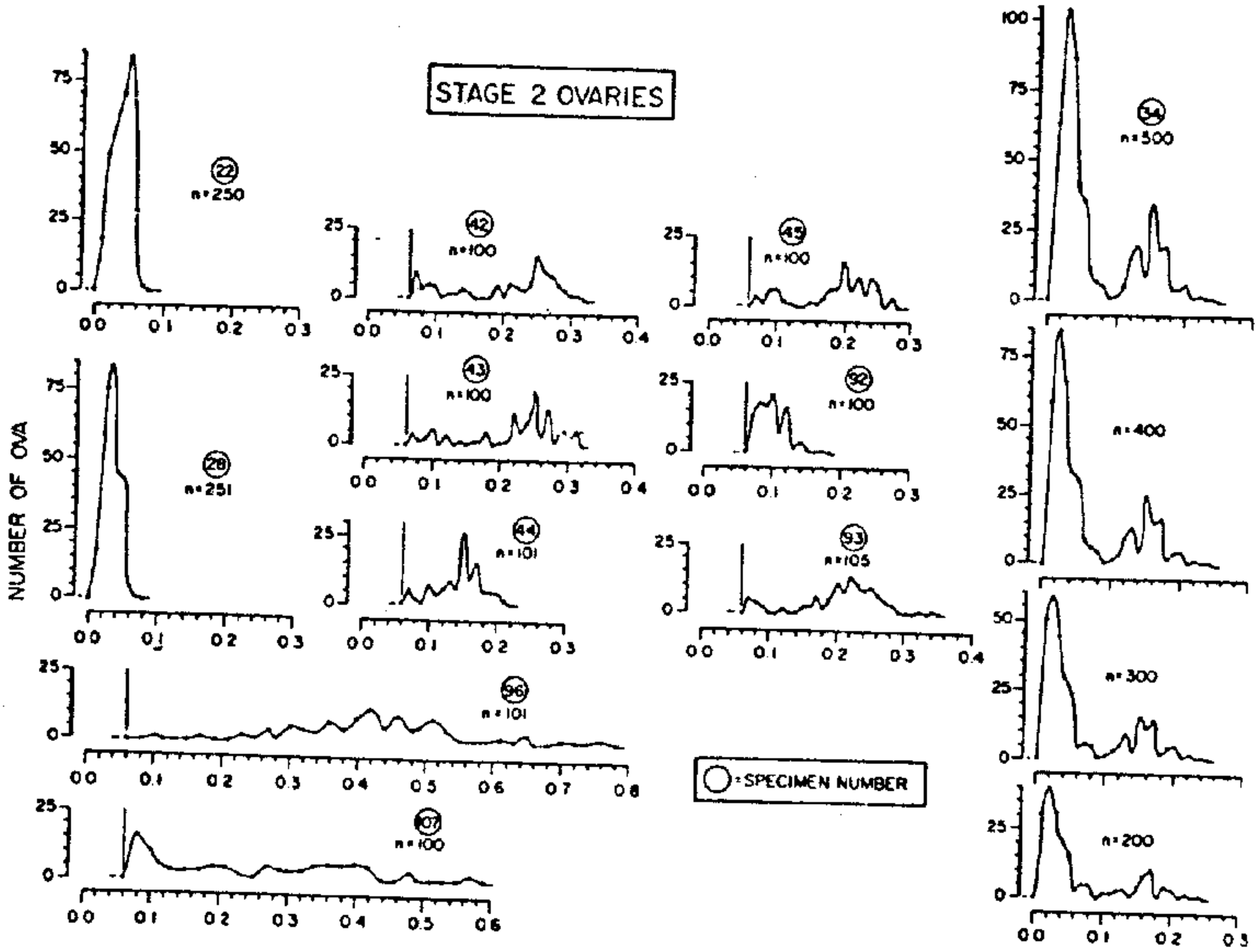

Figure 6. Ovum diameter-frequency distributions of pink shrimp ovary samples classified as stage 2 (developing). Frequency distributions for specimen 34 are based upon sample sizes from 200 to 500 ova.

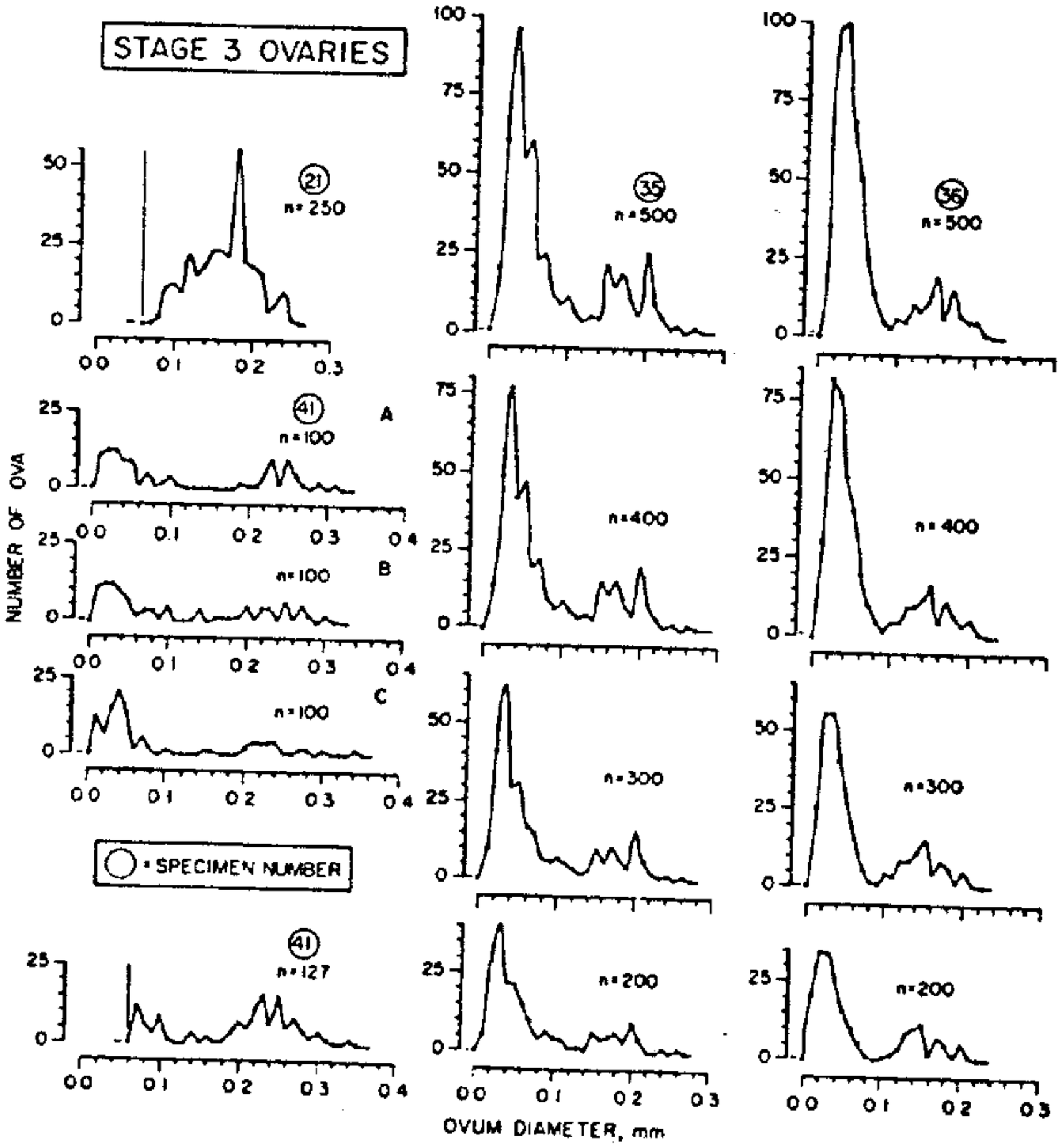

Figure 7. Ovum diameter-frequency distributions of pink shrimp ovary samples classified as stage 3 (nearly ripe). Frequency distributions for specimens 35 and 36 are based upon sample sizes from 200 to 500 ova. For specimen 41, a sample of 100 ova was taken from each of three regions (A-anterior, B-middle, and C-posterior) of the abdominal lobes of the ovary, and the additional sample of 127 ova from the anterior region compares closely with the 100-ovum sample from the same region.

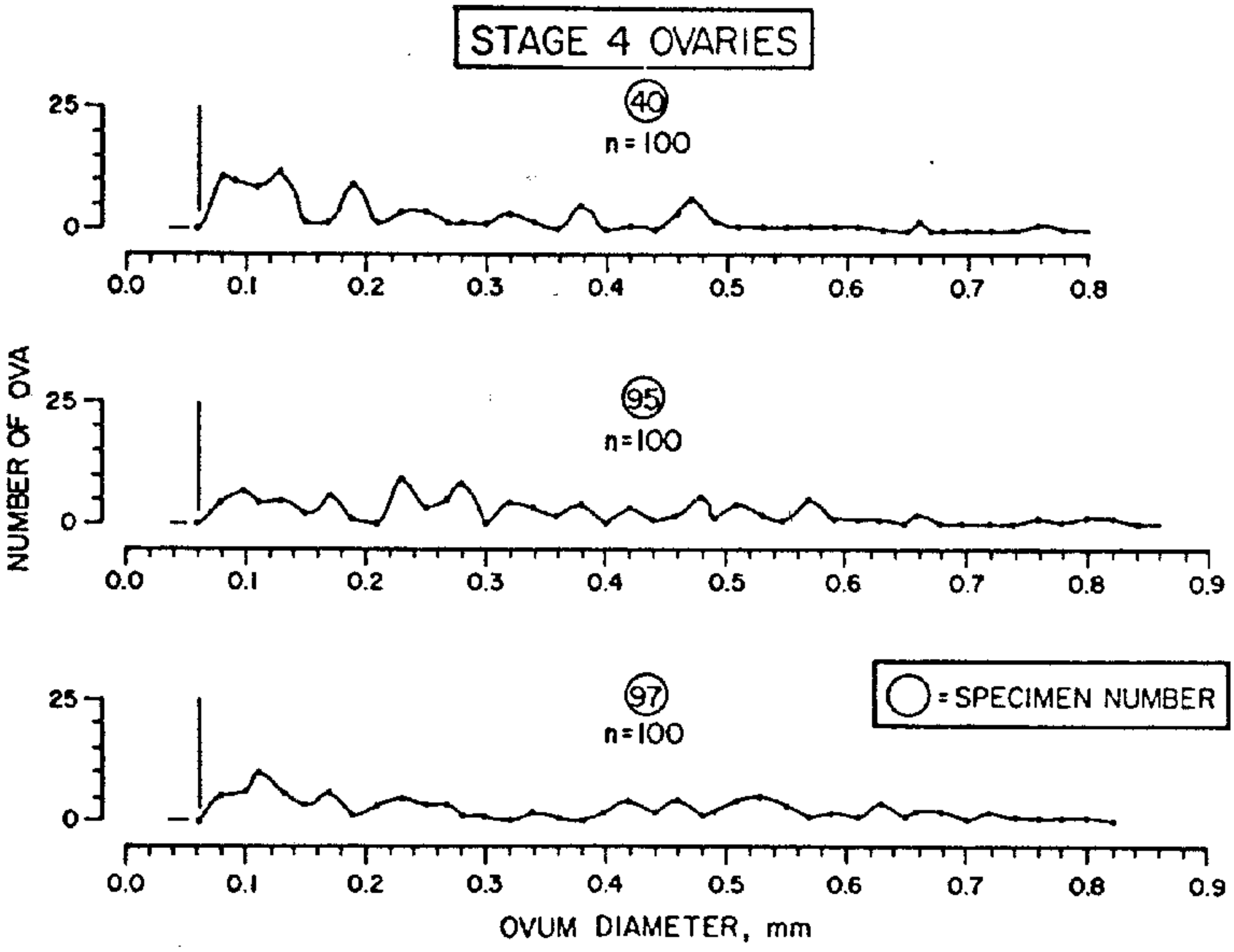

Figure 8. Ovum diameter-frequency distributions of pink shrimp ovary samples classified as stage 4 (ripe).

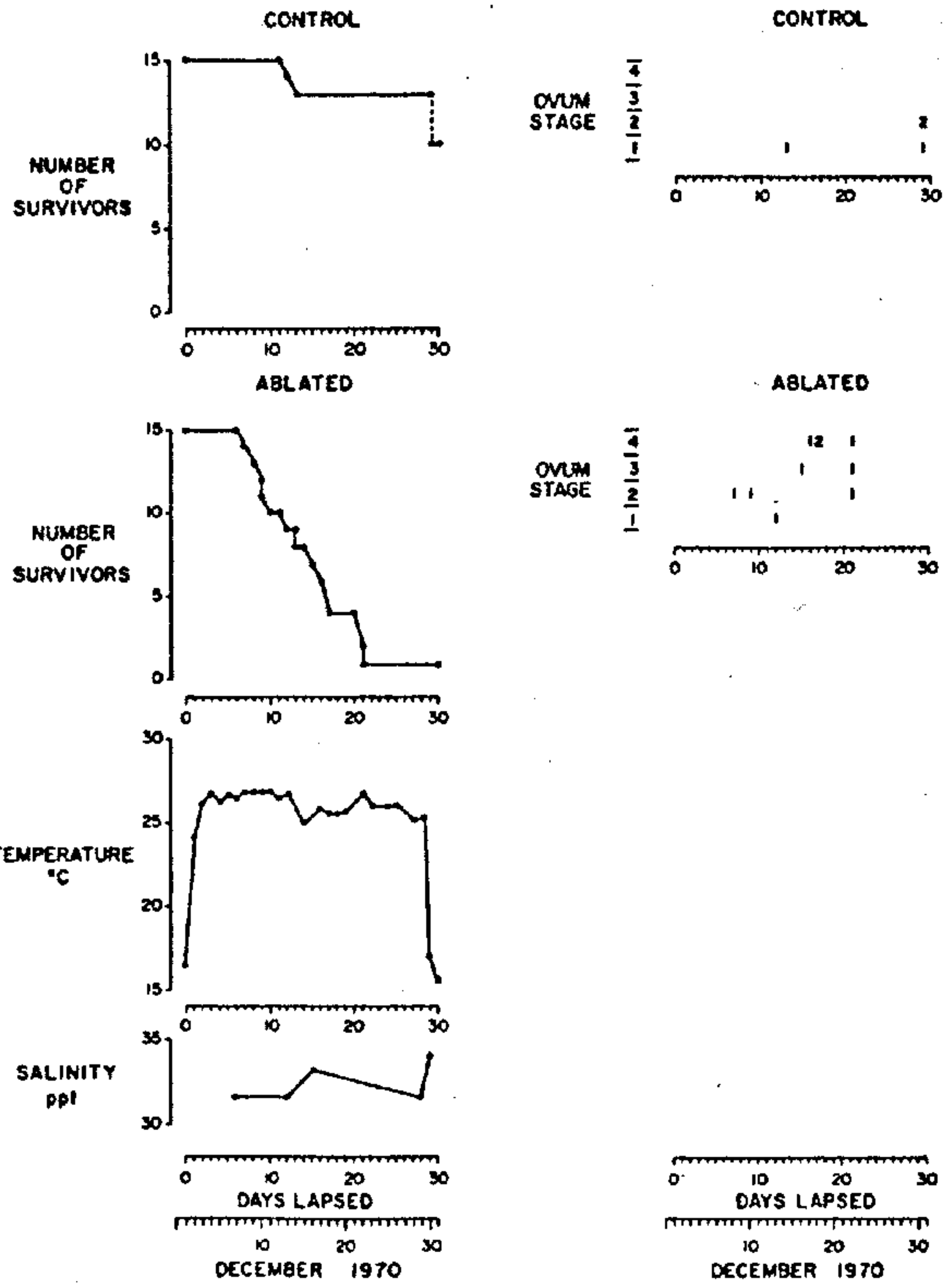

Figure 9. Survival and ovum stage-frequency in an experiment with normal (control) and ablated pink shrimp reared from ova at Turkey Point. The number (frequency) of specimens examined is indicated by both ovum stage and day to show the time course of ovarian maturation. Temperature and salinity are also shown. Lighting (11 hours light: 13 hours darkness) was provided by white flourescent lamps. The shrimp were fed daily with few exceptions. The diet consisted of a mixture of homogenized beef liver, whole egg, squid, Oppenheimer pellets (developed at Florida State University, Tallahassee, especially for shrimp), and Glencoe trout spawner pellets, with calcereous sand filler added to prevent the food from floating. The food was kept frozen until used.

Table 1. - Simple correlation coefficients (r) for the relationships between carapace length (17-45 mm) and two measures of maturation, cross-sectional area of the ovary (0.25-30.5 mm^2) and ovum stage (1-4), and between the two measures of maturation in both normal and ablated female pink shrimp (the number of specimens upon which each coefficient is based is shown in parentheses)[a]

A. Normal Females

	Carapace Length	Ovary Cross-sectional Area	Ovum Stage
Carapace Length	1.00		
Ovary Cross-sectional Area	0.45 (234)	1.00	
Ovum Stage	0.26 (240)	0.50 (235)	1.00

B. Ablated Females

	Carapace Length	Ovary Cross-sectional Area	Ovum Stage
Carapace Length	1.00		
Ovary Cross-sectional Area	0.51 (142)	1.00	
Ovum Stage	0.18 (146)	0.28 (142)	1.00

C. Normal and Ablated Females Combined

	Carapace Length	Ovary Cross-sectional Area	Ovum Stage
Carapace Length	1.00		
Ovary Cross-sectional Area	0.52 (376)	1.00	
Ovum Stage	0.36 (386)	0.55 (377)	1.00

[a]Sample sizes differed because it was not possible to measure all three variables in all 386 specimens.